# Functional Microbial Changes During Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) in Hanford 100H Sediments

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http://esd.lbl.gov/ERT/hanford100h/

## Abstract

To demonstrate the feasibility of a cost-effective remediation technology, using lactate-stimulated bioreduction of dissolved Cr(VI) to form an insoluble mineral precipitation of Cr(III), we conducted bench-scale investigations of sediment samples collected at the Hanford 100H area. Sediments were first saturated with water containing 1000 ppb Cr(VI) and then exposed to sodium lactate, HRC®, HRC Primer, HRC-Extended Release (HRC-X™), Metals Remediation Compound (MRC™) and a no carbon solution. HRC is a polylactate compound with different degrees of polymerization to control its viscosity and solubility in water. MRC is an organosulfur and polylactate combination that reacts directly with Cr, at least initially. In less than 1 week, MRC reduced Cr(VI) to undetectable concentrations, though the abiotic MRC control had only been reduced by 65%. By 3 weeks all the biotic HRC and lactate combinations had undetectable Cr(VI) while the abiotic controls for each had 40-61% of the Cr(VI) remaining. The sediment with no additional carbon source also showed a 64% reduction in Cr(VI) after 3 weeks, whereas the abiotic control had only a 12% reduction in Cr(VI). The HRC compounds performed equally well, while the MRC gave a faster response, although much of this response was abiotic. Phospholipid fatty acid analyses (PLFA), terminal restriction fragment length polymorphisms (TRFLP), clone libraries, direct cell counts, and 16s rDNA microarray analysis demonstrated that the initial densities of microbes is very low (<10⁶ cells/g), but after biostimulation was typically >10⁸ cells/g. Microbial diversity was low but sulfate reducers, *Arthrobacter* spp. and *Geobacter* spp. dominated the samples. The results demonstrate that even in low biomass and diversity environments biostimulation of Cr-reducers can occur and that their functional relationships can be evaluated by various molecular techniques.

## Hypothesis

Lactate (HRC) injection into chromium-contaminated groundwater through an injection well will cause bioreduction of chromate [Cr(VI)] and precipitation of insoluble species of [Cr(III)] on soil particles, probably catalyzed at oxide surfaces at the field scale.

## Objective

To perform laboratory-based investigations to determine the potential for immobilizing and detoxifying chromium contaminated soils and groundwater using bioremediation at the Hanford 100H site.

## Specific Goals

- Determine the background composition of microbial community in soils
- Evaluate the potential for using different types of Hydrogen Release Compounds (HRC) to stimulate
  - microbial biomass in soils
  - reductive precipitation of Cr(VI) to Cr(III)
- Determine changes in microbial populations during HRC stimulated Cr(VI) bioreduction

## Types of Sediments Investigated

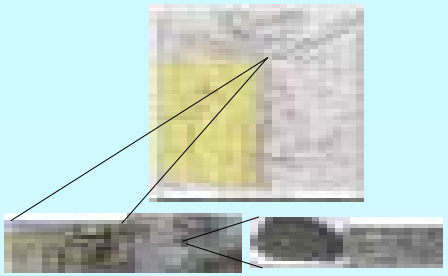
Analyses of sediments collected

- at the Columbia River outcrop
- from two new boreholes the Hanford 100H field site

## General Information about Cr(VI) Biostimulation using lactate/polylactate (HRC™) and MRC

- Hydrogen Release Compound (HRC™):
  - is supplied as an electron donor, like lactate and hydrogen, for microbial production of reducing conditions
  - stimulates microbial reduction and production of species that can chemically reduce Cr(VI) to Cr(III) like Fe(II) and hydrogen sulfide
  - causes the microbial population to remove the oxygen, nitrate, sulfate and other competing electron acceptors, stimulating the transformation of Cr(VI) species to Cr(III) species, which are precipitated on soil particle surfaces.
- Metals Remediation Compound (MRC)
  consists of glycerol tripolylactate and sorbitol cysteinate
  upon microbial degradation releases an organosulfur compound
  irreversibly reacts to produce a metal-organosulfur complex which is sorbed strongly to sediments (Regenesis, 2003)
- Factors affecting Cr(VI) Biostimulation
  - aquifer geochemistry (inorganic common anions and cations, Eh, pH, temperature and DO), nitrate,
  - oxidation conditions caused by recharge of infiltrating water or water from the river and the presence of manganese oxide.

## Sample Collection Map



Hanford formation coarse sample from the mouth of Ringold Coulee, along the White Bluffs

Close-up view of a coarse sample in a sample bag. Notice dark-gray color, angularity, poor sorting and basaltic composition of clasts, all characteristics of the coarse-grained Hanford formation. Penny is for scale.

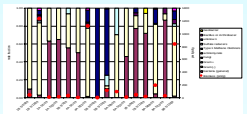
## Types of Analyses to Assess Microbial Populations

- Phospholipid fatty acid analyses (PLFA)
- Terminal restriction fragment length polymorphism (T-RFLP)
- Direct cell counts
- Clone libraries
- Direct cell counts
- 16S rDNA microarray analysis

### Depth of Sediment Samples

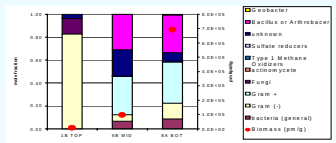
| Sample number | Depth (ft) |
|---|---|
| 1B | 37.5 – 38.0 |
| 2A | 40.5 – 41.0 |
| 3B | 42.0 – 42.5 |
| 4A | 45.0 – 45.5 |
| 5B | 47.0 – 47.5 |
| 6A | 50.0 – 50.5 |
| 7B | 52.0 – 52.5 |
| 8A | 55.0 – 55.5 |
| 9B | 56.5 – 57.0 |

### Sediment microbial community



Phospholipid fatty acid (PLFA) analysis of sediments (Well 699-94-45). Left vertical axis: fractions of constituent microrganisms. Right vertical axis: viable biomass concentration (picomole/g).

### Results of PLFA analysis of R2A enrichment of Hanford sediments

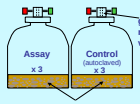



Left vertical axis is a fraction of constituent microorganisms.

Right vertical axis viable biomass concentration, picomole/g.

## Laboratory Microcosm Treatability Study

### Test Schematic




- Gas tight containers with sampling ports
- 100 g Hanford sediment and 33 ml Hanford groundwater amended with:
  - Cr(VI) to 1000 ppb
  - Carbon equivalent to 0.4g C $kg^{-1}$ sediment (~ 1g HRC $kg^{-1}$)
- Autoclaved controls

Types of Tests:

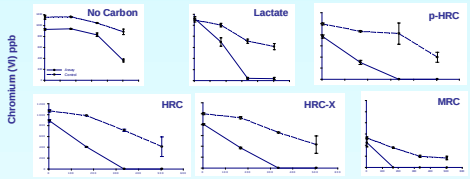
- with and without Carbon (40 mg)
- Lactate
- HRC primer (pHRC) – rapid lactate release formulation
- HRC – original slow release polylactate formulation
- HRC-X – extended release polylactate formulation

HRC compounds release lactate from a polylactate complex at a rate depending on degree of polymerization

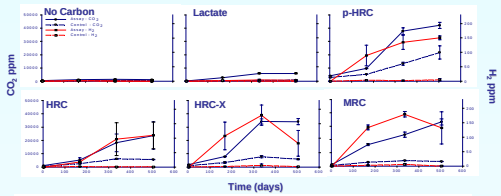
### Chemical analyses of water from Well 699-96-43 used in microcosm simulations

| pH | Cr(VI) | Chloride | Nitrate | Nitrite | Phosphate | Sulfate | DIC | DOC |
|---|---|---|---|---|---|---|---|---|
| 7.1 | 52 ± 1 | 15 ± 6 | 43 ± 3 | <0.25 | <0.25 | 86 ± 16 | 29 ± 0.1 | 1.2 |

### Decrease in Cr(VI) concentration with time as affected by different types of treatment




### Changes in $CO_2$ and $H_2$ concentrations with time as affected by different types of carbon

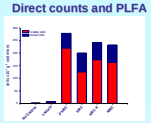


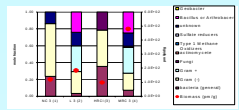

### Test Results

- All carbon applications stimulated Cr(VI) removal
- Majority of removal was caused by biological activity
- Efficacy of Cr(VI) removal by MRC and HRC stimulation
  MRC > HRC = HRC-X = p-HRC
- Cr(VI) still detectable after 3 weeks with Lactate treatment
- $H_2$ headspace concentrations used to determine solution phase $H_2$ at 3 weeks
  No Carbon – 0.12 nM; Lactate – 3 nM; HRC-X – 64 nM;
  HRC – 85 nM; MRC – 104 nM; p-HRC – 118 nM
- Aqueous $H_2$ concentrations of 7-10 nM is associated with methanogenesis;
  1-1.5 nM with sulfate reduction;
  0.2 nM with Fe(III) reduction
  <0.05 nM with Mn(IV)/$NO_3$/Cr(VI) reduction

## Impact of HRC® Products on Microbial Populations During Cr(VI) Removal

### Direct counts and PLFA

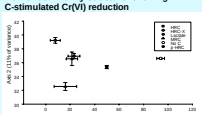




- Bacterial biomass stimulated by added carbon compounds
- HRC products stimulate greater biomass than Na-lactate
- *Arthrobacter/Bacillus* and sulfate reducers stimulated
- PLFA estimate of biomass – underestimate – Hanford coarse material difficult to extract PLFA from
  - analyses being performed again with an initial cell concentration technique developed for low biomass sediments
  - these new extracts will also be analyzed for peaks incorporating $^{13}C$ from $^{13}C$ lactate spikes

### Terminal Restriction Fragment Length Profiling

PCA of community structure following C-stimulated Cr(VI) reduction




- Bacterial community structure is altered by added carbon
- HRC and HRC-X resulted in similar microbial communities
- MRC and p-HRC stimulations resulted in microbial communities most different from originals
- Lactate has no significant effect on bacterial community

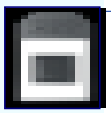
### High density oligonucleotide microarray analyses

**Affymetrix GeneChip**

- Custom-made 16S rDNA array
- 500,000 probes per array
- Detect 9,121 bacteria/archae
- Monitor changes in composition
- Track species dynamics

**Initial results:**

3 samples analyzed – No Carbon – HRC – MRC
Dominant organisms confirmed by 16S clone library analysis



| No Carbon | HRC | MRC |
|---|---|---|
| *Arthrobacter* sp. | *Sporomusa* sp. | *Sporomusa* sp. |
| *Methylophaga* sp. | *Pseudomonas* sp. | *Bacillus* sp. |
| *Cellulomonas* sp. | *Aquaspirillum* sp. | *Burkholderia* sp. |
| *Pseudomonas* sp. | *Acidovorax* sp. | *Pseudomonas* sp. |
| *Blastomonas* sp. | *Clostridium* sp. | *Serratia* sp. |
| *Aquaspirillum* sp. | *Acidobacterium* sp. | *Clostridium* sp. |
| *Acidovorax* sp. | *Cellulomonas* sp. | *Nitrosospira* sp. |
| *Mesorhizobium* sp. | *Mesorhizobium* sp. | *Nitrosomonas* sp. |
| *Micrococcus* sp. | *Arthrobacter* sp. | *Desulfovibacter* sp. |
| *Methylophilus* sp | *Bacillus* sp. | *Fulvimonas* sp. |

## Conclusions

Hanford sediments contain bacterial species:
- capable of Cr(VI) reduction
- tolerant of high concentrations of heavy metals
- capable of metabolism

- All HRC and MRC products:
  - stimulated bacterial biomass and activity
  - enhanced Cr(VI) removal from solution
  - resulted in highly reducing conditions
- 16S rDNA microarrays:
  - identified diverse bacterial communities after stimulation
  - permit ~9,000 bacterial species to be monitored during remediation

## Acknowledgement

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